

Appl. No. 09/721,550
Amdt. dated March 12, 2004
Reply to Final Office action of December 15, 2003

Docket No. 58027-010300

AMENDMENTS TO THE CLAIMS

Please cancel claims 1-40 without prejudice or disclaimer and add new claims 41-71 as follows.

1-40. (canceled)

41. (New) A method for quantifying the amount of a target molecule in solution comprising the steps of:

- a. incorporating one or more fluorescing nucleotide analogs into nucleotide probes to provide fluorescing nucleotide probes;
- b. providing a microarray comprising a substrate having a surface area;
- c. affixing a known number of said fluorescing nucleotide probes onto said substrate
- d. detecting a first level of fluorescence from said fluorescing nucleotide probes disposed upon said substrate;
- e. applying to said substrate a sample solution comprising unlabeled target nucleotide sequences;
- f. providing sufficient conditions and time for unlabeled target molecules to selectively hybridize with fluorescing nucleotide probes on said substrate wherein hybridization of an unlabeled target molecules and fluorescing nucleotide probes quenches fluorescence of said fluorescing nucleotide probes ;
- g. detecting a second level of fluorescence from said fluorescing nucleotide probes disposed upon said substrate after hybridization;
- h. comparing said first and second levels of fluorescence;
- i. repeating steps e through h until quenching of said fluorescing nucleotide probes is reduced until all target molecules are hybridized and no longer quench said fluorescing nucleotide probes ; and
- j. quantifying the number of quenched fluorescing nucleotide probes wherein the number of quenched probes equals the number of target molecules in the sample.

42. (New) The method of claim 41, wherein said fluorescing nucleotide probes are

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comprised of native and nonnative nucleotides.

43. (New) The method of claim 41, wherein the fluorescing nucleotide analogs are nucleotide analogs, including 2-amino purine for adenosine or guanine; ribonucleoside or 2,6-diamino ribonucleoside, formycin A, formycin B, oxyformycin B, toyocamycin, sangivamycin, pseudouridine, showdomycin, minimycin, pyrazomycin, 5-amino-formycin A, 5-amino-formycin B or 5-oxo-formycin A for adenosine; 4-amino-pyrazolo [3,4d] pyrimidine, 4,6-diamino-pyrazolo [3,4d] pyrimidine, 4-oxo-pyrazolo [3,4d] pyrimidine; 4-oxo-6-amino-pyrazolo [3,4d] pyrimidine, 4,6-dioxo-pyrazolo [3,4d] pyrimidine, pyrazolo [3,4d] pyrimidine, 6-amino-pyrazolo [3,4d] pyrimidine or 6-oxo-pyrazolo [3,4d] pyrimidine for cytosine or thymidine.

44. (New) The method of claim 41, wherein said one or more fluorescing nucleotide analogs fluoresces at a wavelength of about 300 nm to about 700 nm.

45. (New) The method of claim 41, wherein said one or more fluorescing nucleotide probes are further comprised of amino acids.

46. (New) The method of claim 41, wherein said one or more fluorescing nucleotide probes are further comprised of carbohydrate.

47. (New) The method of claim 41, wherein said surface area is comprised of quadrants.

48. (New) The method of claim 41, wherein said surface area is comprised of quadrants having different fluorescing nucleotide probe molecules.

47. (New) The method of claim 41, wherein said surface area has from about 100 to about 10,000 different fluorescing nucleotide probe molecules located upon about 100 to about 10,000 different quadrants.

48. (New) The method of claim 47, said surface has about 100 to about 1,000 fluorescing nucleotide probe molecules per quadrant.

49. (New) A method for quantifying the amount of a target molecule in solution

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comprising the steps of:

- a. incorporating one or more fluorescing nucleotide analogs into nucleotide probes to provide fluorescing nucleotide probes;
- b. providing a first substrate having a surface area;
- c. affixing a known number of said fluorescing nucleotide probes onto the substrate;
- d. detecting a first level of fluorescence from said fluorescing nucleotide probes on the substrate;
- e. contacting said first substrate with a sample solution comprising unlabeled target nucleotide sequences;
- f. providing sufficient conditions and time for unlabeled target molecules to selectively hybridize with fluorescing nucleotide probes on said substrate wherein hybridization of an unlabeled target molecule and an fluorescing nucleotide probe quenches fluorescence from said fluorescing nucleotide probe;
- g. removing the first substrate and detecting a second level of fluorescence from said fluorescing nucleotide probes after hybridization;
- h. repeating steps a. through g with subsequent substrates, having surface areas comprising known numbers of fluorescing nucleotide probes until all target molecules are hybridized and no longer quench said fluorescing nucleotide probes; and
- i. quantifying the amount of target molecule in the sample solution by adding the known number of fluorescing nucleotide probes present on the first substrate and subsequent substrates contacted with and quenched by the unlabeled target molecule whereby the amount of the target molecule is quantified.

50. (New) The method of claim 49, wherein said fluorescing nucleotide probes are comprised of native and nonnative nucleotides.

51. (New) The method of claim 49, wherein the fluorescing nucleotide analogs are nucleotide analogs, including 2-amino purine for adenosine or guanine; ribonucleoside or 2,6-diamino ribonucleoside, formycin A, formycin B, oxyformycin B, toyocamycin, sangivamycin, pseudoouridine, showdomycin, minimycin, pyrazomycin, 5-amino-formycin A, 5-amino-formycin B or 5-oxo-formycin A for adenosine; 4-amino-pyrazolo [3,4d] pyrimidine, 4,6-

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diamino-pyrazolo [3,4d] pyrimidine, 4-oxo-pyrazolo [3,4d] pyrimidine; 4-oxo-6-amino-pyrazolo [3,4d] pyrimidine, 4,6-dioxo-pyrazolo [3,4d] pyrimidine, pyrazolo [3,4d] pyrimidine, 6-amino-pyrazolo [3,4d] pyrimidine or 6-oxo-pyrazolo [3,4d] pyrimidine for cytosine or thymidine.

52. (New) The method of claim 49, wherein said one or more fluorescing nucleotide analogs fluoresces at a wavelength of about 300 nm to about 700 nm.

53. (New) The method of claim 49, wherein said fluorescing nucleotide probes are further comprised of amino acids.

54. (New) The method of claim 49, wherein said fluorescing nucleotide probes are further comprised of carbohydrate.

55. (New) The method of claim 49, wherein said surface area has from about 100 to about 10,000 different fluorescing nucleotide probe molecules.

56. (New) The method of claim 49, wherein the substrate is a bead.

57. (New) The method of claim 56, wherein said bead size ranges from about 10 microns to about 20 microns.

57. (New) The method of claim 56, wherein the bead is formed of a ferromagnetic metal core and a polymeric coating.

58. (New) The method of claim 56 having from about 100 to about 1,000 labeled fluorescing nucleotide probe molecules attached to the surface area of the bead.

59. (New) The method for quantifying the amount of a target molecule in a sample solution comprising the steps of:

- a. incorporating a nucleotide analog including 2-aminopurine into nucleotide probes to provide fluorescing nucleotide probes;
- b. affixing a known number of said the fluorescing probes onto a substrate;
- c. detecting a first level of fluorescence from said fluorescing nucleotide probes on the substrate;

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- d. contacting said substrate with said sample solution containing unlabeled target molecules;
- e. providing sufficient conditions and time for unlabeled target molecules in said solution to selectively pair and hybridize with said fluorescing nucleotide probes affixed on said substrate wherein hybridization of an unlabeled target molecule and fluorescing probe quenches fluorescence of the nucleotide probes;
- f. removing said substrate from the solution and detecting a second level of fluorescence from the fluorescing nucleotide probes on the substrate;
- g. comparing said first and second level of fluorescence;
- h. repeating steps d. through g. by re-contacting said sample solution with said substrate or additional substrates having a known number of fluorescing nucleotide probes until target molecules no longer quench the fluorescence from said fluorescing probes; and
- i. quantifying the amount of target molecules by determining the number of quenched fluorescing probes.

60. (New) The method of claim 59, wherein said fluorescing nucleotide probes are comprised of native and nonnative nucleotides.

61. (New) The method of claim 59, wherein the fluorescing nucleotide probes are comprised of carbohydrate.

62. (New) The method of claim 59 wherein the fluorescing nucleotide probe molecules are comprised of amino acids.

63. (New) The method of claim 59 wherein the substrate is a microarray further having the surface area divided into quadrants wherein each different quadrant has different fluorescing nucleotide probe molecules.

64. (New) The method of claim 59, having from about 100 to about 10,000 different fluorescing nucleotide probe molecules located upon about 100 to about 10,000 different quadrants.

65. (New) The method of claim 59, wherein the substrate is a microarray further

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having the surface area divided into quadrants and having about 100 to about 1,000 fluorescing nucleotide probe molecules per quadrant.

66. (New) The method of claim 59, wherein the substrate is a bead.

67. (New) The method of claim 66, wherein said bead size ranges from about 10 microns to about 20 microns.

68. (New) The method of claim 66, wherein the bead is formed of a ferromagnetic metal core and a polymeric coating.

69. (New) The method of claim 66, having from about 100 to about 1,000 fluorescing nucleotide probe molecules attached to the surface area of the bead.

70. (New) The method of claim 66, wherein the level of label expression is evaluated using a flow cytometer.

71. (New) The method of claim 66, wherein the second level is significantly lower than the first level and said second levels of fluorescence approach zero and/or about background levels.